



UNITED STATES DEPARTMENT OF COMMERCE  
PATENT AND TRADEMARK OFFICE

Inventors : Aki Kobayashi et al. )  
Serial No. : 10/500,355 ) ART UNIT: 1617  
Filing Date : June 30<sup>th</sup> 2004 ) EXAMINER:

Ms. Kim M Jennifer

Title : Antiseptic disinfectant, and cosmetics and toiletries, medicine  
or food containing the same

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

DECLARATION

1. We, Fumihiro Okada and Hiroya Okamoto, hereby declare as follows.

2

(1). I, Fumihiro Okada, was born on February 11<sup>th</sup>, 1966, and graduated from Chemistry Faculty of Science and engineering, Ritsumeikan University in March, 1989, and earned doctorate in Science as "No. 041 of Osaka Prefecture University".

(2) My backgrounds from the graduation to the present are following.

- 1989, April 1<sup>st</sup> Research worker in Development Research Laboratories of MANDOM CORPORATION
- 1997, April 1<sup>st</sup> Section head in Fundament Research Laboratories of MANDOM CORPORATION
- 2002, April 1<sup>st</sup> to date laboratory head in Quality Estimation Laboratories of MANDOM CORPORATION
- 2004, April 1<sup>st</sup> to date Visiting associate professor in Japan Advance Institute of Science And Technology

(3) Societies which I belong to are following.

- 1990, April The society for antibacterial and antifungal agents, Japan as a member (as a member of editorial board of the society from 2004, April to date, as a councilor from 2007, January as well)
- 1991, April to date Japanese Society for Bacteriology as a member
- 1994, April to date The Japanese Biochemical Society as a member
- 1999, May to date The Society of Cosmetic Chemists of Japan as a committee member
- 2001, May to date Japanese Society for contact dermatitis as a representative member

(4) Papers I presented are following.

(Academic paper)

1. Preservative Evaluation of Cosmetics and Toiletries by Microbial Calorimetry / Okada, F., Fujiwara, N., Matsuyama, K., Takahashi, K. / *J. Soc. Cosmet. Chem. Jpn.*, 27(3), 242-248 (1993)
2. Simplified, Time-saving Microbial Tests for Cosmetics and Toiletries / Okada, F. / *J. Soc. Cosmet. Chem. Jpn.*, 32(2), 131-139 (1998)
3. Quantitative Analysis of Antimicrobial Actions of Drugs Studied by Microbial Calorimetry / Okada, F. / *Netsu Sokutei.*, 25(4), 132-137 (1998)
4. Microbial Calorimetry of Supported Cultures and Its Application to the Study of Antimicrobial Action / Okada, F., Kobayashi, A., Fujiwara, N., Takahashi, K. / *Biocontrol Science*, 3, pp.79-85 (1998)
5. Bacteriostatic and Bactericidal Actions of Antimicrobial Drugs Studies by Microbial Calorimetry / Okada, F., Kobayashi, A., Fujiwara, N., Takahashi, K. / *Biocontrol Science*, 4, pp.35-39 (1999)
6. Calorimetric Analysis of Antimicrobial Effect of *p*-hydroxybenzoic Acid Alkyl Esters / Okada, F., Kobayashi, A., Fujiwara, N., Arimoto, N., Takahashi, K. / *Biocontrol Science*, 4, pp.67-73 (1999)
7. Calorimetric Study of the Antimicrobial Action of Various Polyols Used for Cosmetics and Toiletries / Aono, A., Takahashi, K., Mori, N., Shimizu, H., Kobayashi, A., Fujiwara, N., Okada, F. / *Netsu Sokutei.*, 26(1), 2-8 (1999)
8. Various materials used in Cosmetics and Toiletries, or medicine, having antibacterial effects / Okamoto, H., Kobayashi, A., Okada, F. / "Course relating to microorganism, and antiseptic agent or disinfecting agent used in cosmetics and toiletries, or medicine" in Journal of The society for

1 antibacterial and antifungal agents, Japan 28, (12) , 801-810 (2000)

- 2 9. Solution to Environmental Problems on Formulation Development of  
3 Cosmetics and Toiletries / Okada, F. / FRAGRANCE JOURNAL, 5, 47-50  
4 (2003)

5  
6 (Books)

- 7 1. Materials having antiseptic disinfectant effects / Okada, F. / Microbial  
8 contamination Protection and Antiseptic Design Engineering for Cosmetics  
9 and Toiletries external preparation, Technical Information Institute Co.,  
10 Ltd, pp.147-157 (2001)

1 3.

2 (1) I, Hiroya Okamoto, was born on November 27<sup>th</sup>, 1969, and graduated from  
3 applied biology department of fiber faculty, Kyoto Institute of Technology in  
4 March, 1993.

5  
6 (2) My backgrounds from the graduation to the present are following.

- 7 • 1993, April 1<sup>st</sup> Research worker in Product Development Research  
8 Laboratories of MANDOM CORPORATION  
9 • 2004, April 1<sup>st</sup> to date Section head in Quality Estimation  
10 Laboratories of MANDOM CORPORATION  
11

12 (3) Papers I presented are following.

- 13 1. Various materials used in Cosmetics and Toiletries, or medicine, having  
14 antibacterial effects / Okamoto, H., Kobayashi, A., Okada, F, / "Course  
15 relating to microorganism, and antiseptic agent or disinfecting agent used  
16 in cosmetics and toiletries, or medicine" in Journal of The society for  
17 antibacterial and antifungal agents, Japan 28, (12) , 801-810 (2000)  
18 2. Application of 1,2-alkanediol having antibacterial effects on Cosmetics and  
19 Toiletries / Okamoto, H. / FRAGRANCE JOURNAL, 4, 34-38 (2006)  
20 3. Current development trend of adaphoretic and deodorant products for men  
21 / Okamoto, H., Endo, Y., Kasahara, K. / FRAGRANCE JOURNAL, 5, 38-42  
22 (2006)  
23

1 4. We are each one of the inventors of US patent application Serial No.  
2 10/500,355. "Antiseptic disinfectant, and cosmetics and toiletries, medicine  
3 or food containing the same", and have a full knowledge regarding the contents  
4 thereof.

5  
6 5. Hereinafter, we would like to present the test results obtained from the side by  
7 side comparison with the closest prior art.

8 [Object]

9 The object is to confirm whether perfumes including Carvacrol or  
10 Limonene which is described in the Kim et al. (J. Agric. Food Chem. 1995, 43,  
11 2839-2845) and other essential oil or extract known as antibacterial compounds  
12 enhance the antibacterial activity that 1,2-alkanediol originally has against a  
13 broad range of strain by combining those with 1,2-alaknediol.

14  
15 [Method]

16 The test was performed according to the method described in US patent  
17 application "Serial No. 10/500,355. Thus, the method is as bellow.

18 (Selection of samples)

19 Among Perfumes, Farnesol,  $\alpha$ -Bisabolol, Limonene, Camphor, Carvacrol  
20 and Hinokitiol were selected, and then tested.

21 Among the essential oil or extract known as having antibacterial activity,  
22 Citronella oil, Eucalyptus oil, Basil oil and striped bamboo extract were selected,  
23 and then tested.

24 (Sample bacterias)

25 Pseudomonas aeruginosa IFO13275 (Pseudomonas) as Gram-negative  
26 bacteria, Staphylococcus aureus IFO13276 (Staphylococcus aureus) as  
27 Gram-positive bacteria, Candida albicans IFO1594 (mycotic stomatitis) as yeast  
28 and Aspergillus niger (Black mold) as Fungus were used as sample bacterias.

29 (Preparation of bacteria solution)

30 For preparing bacteria solution, as to Pseudomonas and Staphylococcus  
31 aureus, they were incubated at 35 °C in the agar medium, and further incubated  
32 at 35 °C. after transferred into the bouillon medium. The inoculating bacteria  
33 were prepared by diluting the obtained culture solution to about  $10^8$  inoculating  
34 cell /ml.

35 As to yeast (Candida albicans), the inoculating bacteria were prepared by  
36 incubating the yeast in the same way as Pseudomonas and Staphylococcus

1 aureus at 30 °C. and diluting the yeast to about  $10^7$  cell /ml.

2 As to Fungus, the inoculating bacteria were prepared by incubating the  
3 fungus at 25 °C and suspending its sporozoite into the physiological saline with  
4 2% of Tween 80 (polyoxyethylene(20)sorbitan Monoleate) and diluting it to about  
5  $10^6$  cell /ml..

6 (Preparation of Diluting Series of the Test Material)

7 With a dilution solvent of ethyl cellosolve of 20 w/w %, 1,2-octanediol  
8 solution of 5, 4, 3, 2.5, 2.25, 2, 1.75, 1.5, 1.25 and 1 w/v % were prepared.

9 While, as to the samples such as the perfume, essential oil or extract, and  
10 as to the 50:50 by weight mixture of 1,2-octanediol and the samples, the dilution  
11 series were prepared by doubling diluting the 20w/v % solution.

12 (Measurement of Minimum Inhibitory Concentration (MIC))

13 1 ml of each of the above-mentioned dilution series including the test  
14 material and 9 ml of the agar medium were introduced to each schales, and the  
15 above-mentioned inoculating bacteria was applied on each of the schales at the  
16 length of 1 cm.

17 Pseudomonas and Staphylococcus aureus were incubated at 35 °C., and it  
18 was judged whether or not the bacteria had grown two days later. The yeast and  
19 fungus were incubated at 25 °C., and it was judged whether or not the bacteria  
20 had grown three days later. The minimum concentration in which the bacteria  
21 had grown was MIC.

22 Still more, antibacterial activity can be evaluated by the MIC. When the  
23 concentration of the test material is low, it does not affect the microbes. As the  
24 concentration gets higher, growth inhibition occurs. Further, as the  
25 concentration gets higher, the growth inhibition progresses, eventually to stop  
26 growth. The concentration at the final point is shown as MIC. Accordingly, when  
27 the concentration goes above MIC, microbes die.

28  
29 [Result]

30 MIC on the each sample is shown in the next page. (See the Table. 1 to  
31 Table. 10)

(Table 1) The minimum concentration for Farnesol (µg/mL)

	P.aeruginosa	S.aureus	C.albicans	A.niger
1,2-Octanediol	3000	2250	1500	1250
Farnesol	5000	78	5000	5000
50:50 by weight mixture of the two	5000	313	5000	2500
1,2-Octanediol therein	2500	156	2500	1250
Farnesol therein	2500	156	2500	1250

(Table 2) The minimum concentration for α-Bisabolol (µg/mL)

	P.aeruginosa	S.aureus	C.albicans	A.niger
1,2-Octanediol	3000	2250	1500	1250
α-Bisabolol	5000	625	5000	5000
50:50 by weight mixture of the two	5000	1250	5000	2500
1,2-Octanediol therein	2500	625	2500	1250
α-Bisabolol	2500	625	2500	1250

(Table3) The minimum concentration for Limonene (µg/mL)

	P.aeruginosa	S.aureus	C.albicans	A.niger
1,2-Octanediol	3000	2250	1500	1250
Limonene	5000	5000	5000	5000
50:50 by weight mixture of the two	5000	5000	5000	2500
1,2-Octanediol therein	2500	2500	2500	1250
Limonene therein	2500	2500	2500	1250

(Table 4) The minimum concentration for Camphor (µg/mL)

	P.aeruginosa	S.aureus	C.albicans	A.niger
1,2-Octanediol	3000	2250	1500	1250
Camphor	5000	5000	5000	5000
50:50 by weight mixture of the two	5000	5000	5000	2500
1,2-Octanediol therein	2500	2500	2500	1250
Camphor therein	2500	2500	2500	1250

(Table 5) The minimum concentration for Carvacrol ( $\mu\text{g/mL}$ )

	<i>P.aeruginosa</i>	<i>S.aureus</i>	<i>C.albicans</i>	<i>A.niger</i>
1,2-Octanediol	3000	2250	1500	1250
Carvacrol	2500	313	313	625
50:50 by weight mixture of the two	2500	625	313	1250
1,2-Octanediol therein	1250	313	156	625
Carvacrol therein	1250	313	156	625

(Table 6) The minimum concentration for Hinokitiol ( $\mu\text{g/mL}$ )

	<i>P.aeruginosa</i>	<i>S.aureus</i>	<i>C.albicans</i>	<i>A.niger</i>
1,2-Octanediol	3000	2250	1500	1250
Hinokitiol	250	125	32	32
50:50 by weight mixture of the two	5000	2500	2500	1250
1,2-Octanediol therein	2500	1250	1250	625
Hinokitiol therein	2500	1250	1250	625

(Table 7) The minimum concentration for Citronella oil ( $\mu\text{g/mL}$ )

	<i>P.aeruginosa</i>	<i>S.aureus</i>	<i>C.albicans</i>	<i>A.niger</i>
1,2-Octanediol	3000	2250	1500	1250
Citronella oil	5000	1250	1250	1250
50:50 by weight mixture of the two	5000	2500	2500	1250
1,2-Octanediol therein	2500	1250	1250	625
Citronella oil therein	2500	1250	1250	625

(Table 8) The minimum concentration for Eucalyptus oil ( $\mu\text{g/mL}$ )

	<i>P.aeruginosa</i>	<i>S.aureus</i>	<i>C.albicans</i>	<i>A.niger</i>
1,2-Octanediol	3000	2250	1500	1250
Eucalyptus oil	5000	5000	5000	5000
50:50 by weight mixture of the two	5000	5000	5000	2500
1,2-Octanediol therein	2500	2500	2500	1250
Eucalyptus oil therein	2500	2500	2500	1250



(Table 9) The minimum concentration for Basil oil ( $\mu\text{g/mL}$ )

	<i>P.aeruginosa</i>	<i>S.aureus</i>	<i>C.albicans</i>	<i>A.niger</i>
1,2-Octanediol	3000	2250	1500	1250
Basil oil	5000	5000	5000	5000
50:50 by weight mixture of the two	5000	5000	2500	2500
1,2-Octanediol therein	2500	2500	1250	1250
Basil oil therein	2500	2500	1250	1250

(Table 10) The minimum concentration for Striped bamboo extract ( $\mu\text{g/mL}$ )

	<i>P.aeruginosa</i>	<i>S.aureus</i>	<i>C.albicans</i>	<i>A.niger</i>
1,2-Octanediol	3000	2250	1500	1250
Striped bamboo extract	5000	5000	5000	5000
50:50 by weight mixture of the two	5000	5000	2500	2500
1,2-Octanediol therein	2500	2500	1250	1250
Striped bamboo extract therein	2500	2500	1250	1250

Next, the obtained each MIC of 1,2-octanediol, the samples such as perfume or essential oil, and the 50:50 mixture by weight of 1,2-octanediol and the samples corresponding the compounding amount of 1,2-octanediol and the samples was plotted to make out the dual minimum inhibitory concentration diagram. Action and effect in case of that two kinds of antibacterial materials are compounded can be judged by the dual minimum inhibitory concentration diagram.

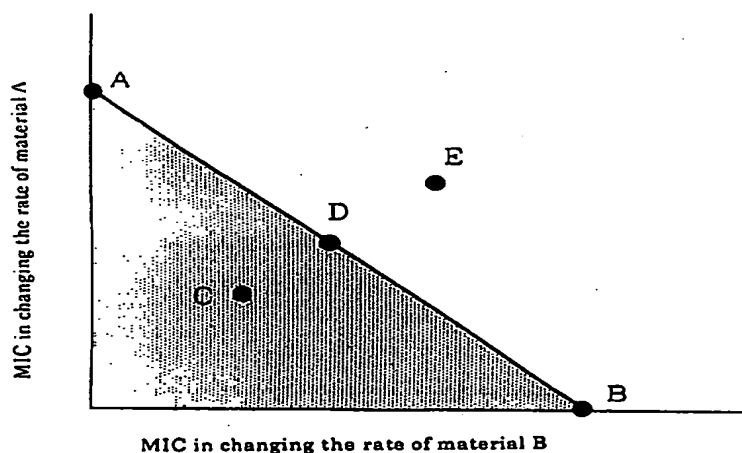
In this page, we would like to explain about evaluation of minimum inhibitory concentration (MIC) using dual minimum inhibitory concentration diagram, which is also described in the present specification.

#### (Evaluation of MIC)

The actions caused by compounding two antibacterial materials are roughly classified into synergistic action, additive action and counteraction. Synergistic action is the action in that two kinds of agent act synergistically, to enhance the antibacterial activity that the agents originally have. The additive action is the action in that the antibacterial activities of the agents are put together. The counteraction is the action in that one agent cancels the antibacterial activity of the other agent.

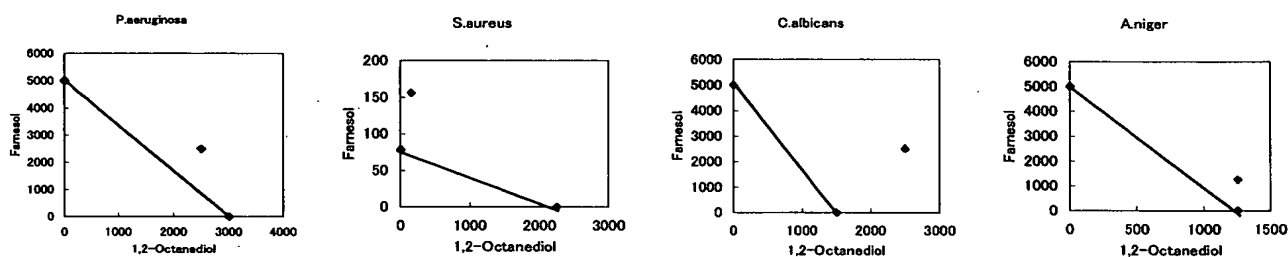
The method using dual minimum inhibitory concentration, as shown in Fig. 1-A for example, is the method to measure MICs as to material A and Material B at different proportions of them and to judge the result in view of the graph. In this method, a line is drawn to connect MIC (point A) in using only material A to MIC (point B) in using only material B. When the MIC (point C) in using both materials is inside the line, it is judged to be Synergistic action that antibacterial activity was strengthened by the combined use. When the MIC (point D) is on the line, it can be judged to be the additive action. When the MIC (point E) is in the outside of the line, it is judged to be the counteraction that cancels antibacterial activity of one or both of the materials to decrease the antibacterial activity.

(Fig 1-A) Diagram showing an example of a method to determine the effect of compounding two antibacterial materials by means of dual minimum inhibitory concentration

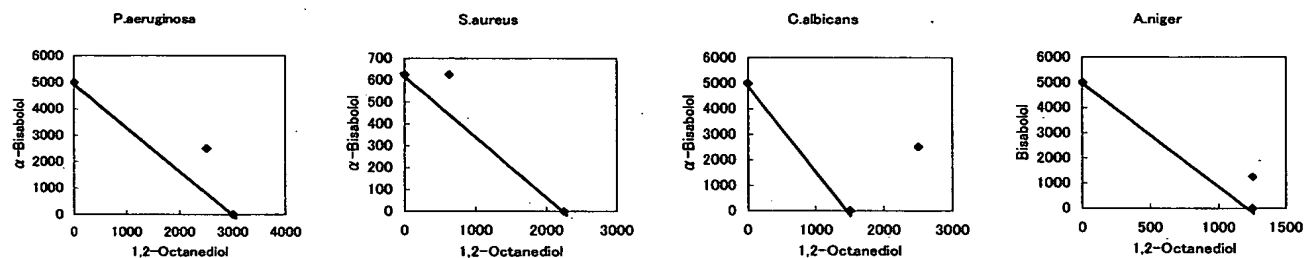


Back to the outstanding test result, the dual minimum inhibitory concentration diagram as to the each samples are shown as below. (See the Fig. 1 to Fig. 10) In the each Figs, the diagrams for *P.aeruginosa*, *S.aureus*, *C.albicans* and *A.niger* appear from left to right in that order

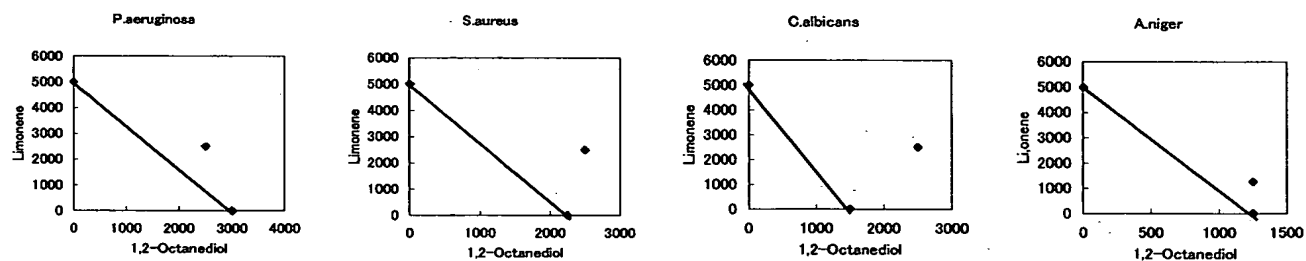
(Fig. 1) The dual minimum inhibitory concentration diagram for Farnesol



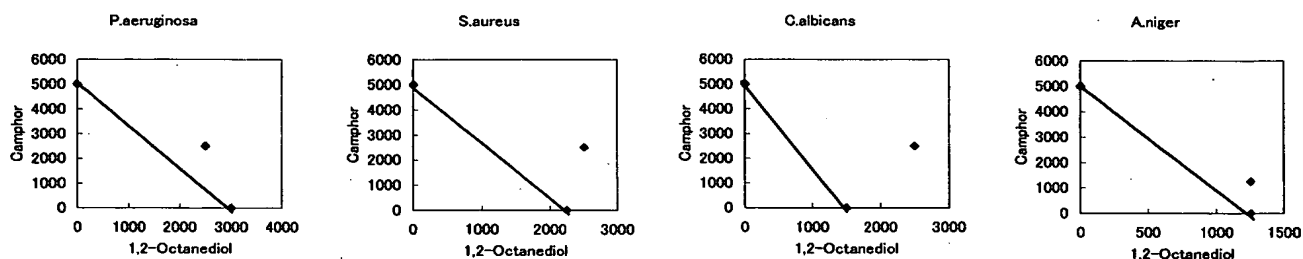
(Fig. 2) The dual minimum inhibitory concentration diagram for  $\alpha$ -Bisabolol



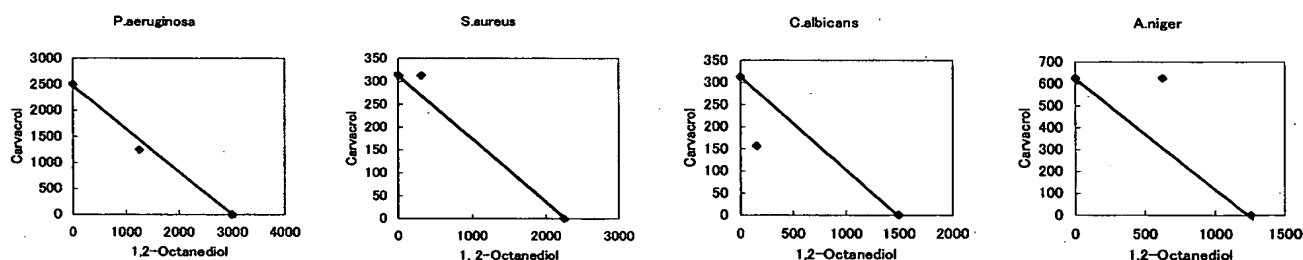
(Fig. 3) The dual minimum inhibitory concentration diagram for Limonene



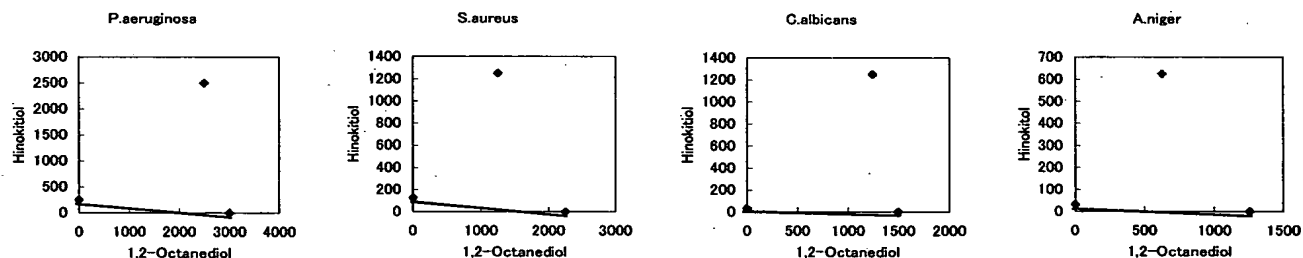
1 (Fig. 4) The dual minimum inhibitory concentration diagram for Camphor



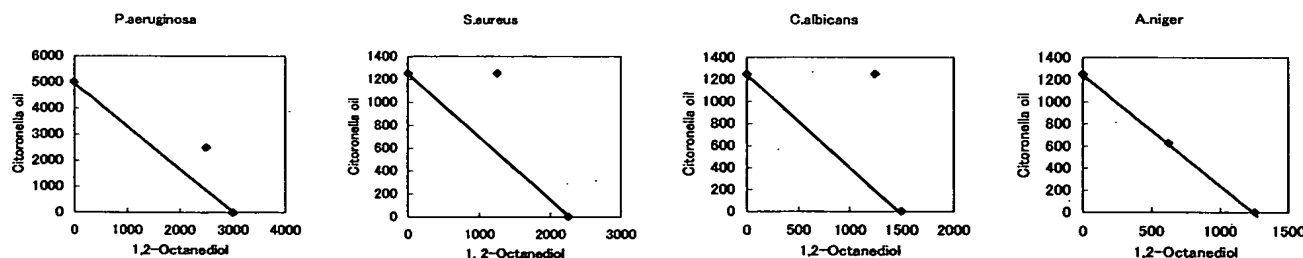
2 (Fig. 5) The dual minimum inhibitory concentration diagram for Carvacrol



3 (Fig. 6) The dual minimum inhibitory concentration diagram for Hinokitiol



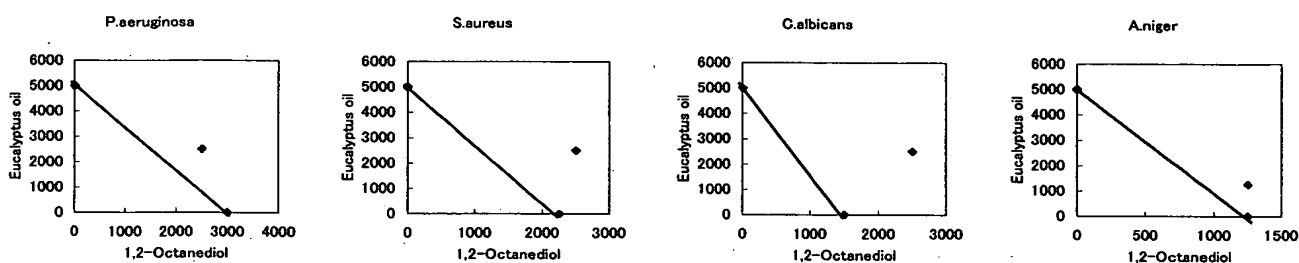
4 (Fig. 7) The dual minimum inhibitory concentration diagram for Citronella oil



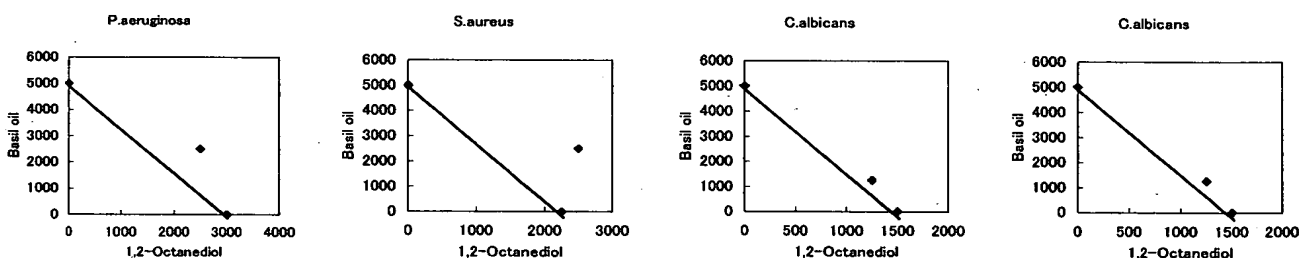
5

6

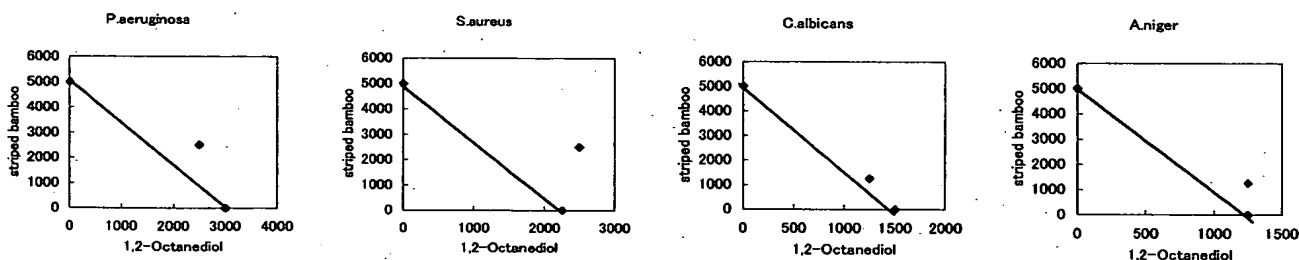
(Fig. 8) The dual minimum inhibitory concentration diagram for Eucalyptus oil



(Fig. 9) The dual minimum inhibitory concentration diagram for Basil oil



(Fig. 10) The dual minimum inhibitory concentration diagram for Striped bamboo extract



Further, according to the dual minimum inhibitory concentration diagram of Fig. 1 to Fig. 10, how antibacterial activity obtained by combination of the 1,2-octanediol and each examples is determined by the following evaluation criterion. The evaluation results are shown in the Table 11.

○: There is synergistic action in the antibacterial effect.

△: There is additive action in the antibacterial effect.

×: There is counteraction in the antibacterial effect.

(Table 11)

Samples	Fig.	<i>P.aeruginosa</i>	<i>S.aureus</i>	<i>C.albicans</i>	<i>A.niger</i>
Farnesol	1	×	×	×	×
$\alpha$ -Bisabolol	2	×	×	×	×
Limonene	3	×	×	×	×
Camphor	4	×	×	×	×
Carvacrol	5	△	×	○	×
Hinokitiol	6	×	×	×	×
Citronella oil	7	×	×	×	△
Eucalyptus oil	8	×	×	×	×
Basil oil	9	×	×	△	△
Striped bamboo extract	10	×	×	△	×

#### [Consideration]

We selected Carvacrol and Limonene, which are described in the Kim et al. (J. Agric. Food Chem. 1995, 43, 2839-2845), Farnesol,  $\alpha$ -Bisabolol, Camphor and Hinokitiol as the sample perfume to be tested. Further, we selected Citronella oil, Eucalyptus oil, Basil oil and striped bamboo extract as the sample essential oil or extract to be tested. These are all known as having antibacterial activity and tested by combining with 1,2-alkanediol to confirm whether the combinations yield synergistic action in their antibacterial effect or not.

In the above-test, all tested perfumes, essential oils or extracts except for Carvacrol showed counteraction or additive action against Gram-negative bacteria, Gram-positive bacteria, yeast and Fungus, when the samples were combined with 1,2-octanediol. As for Carvacrol, it shows counteraction against *S.aureus* and *A.niger*, and additive action against *P.aeruginosa*.

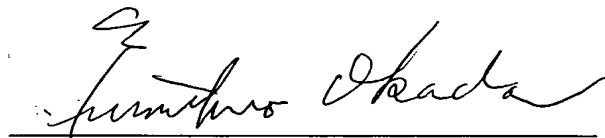
Finally, by comparing the examples in the present specification of this case with the test result on this declaration, we are able to conclude that only particular perfumes can enhance antibacterial activity that 1,2-alkanediol originally has, against a broad range of strains, thus only particular perfumes such as those indicated in the claims 1-12 according to the present invention show synergistic action with 1,2-alkanediol.

In conclusion, because such synergistic effect obtained from combinations of such particular perfumes and 1,2-alkanediol is unexpected, and therefore can

1 not be readily assumed by person in the art.  
2

3 6. We further declare that all statements made herein of our own knowledge are  
4 true and that all statements made on information and belief are believed to be  
5 true.  
6

7  
8  
9  
10 Date: June 12, 2007



Fumihiko Okada

11  
12  
13  
14  
15  
16  
17 Date: June 12, 2007



Hiroya Okamoto